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Comparison of Sampling Methods for the Detection of *Salmonella* on Whole Broiler Carcasses Purchased from Retail Outlets

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ABSTRACT

An experiment was conducted to compare the effectiveness levels of two methods in recovering *Salmonella* from the same carcass. One hundred fresh whole broiler chickens were purchased from retail outlets over a 5-week period (20 carcasses per week). After carcasses had been aseptically removed from the packages and giblets had been removed, the carcasses were placed in sterile bags containing 400 ml of 1% buffered peptone water, the bags were shaken for 60 s, and a 30-ml aliquot was removed and incubated for 24 h at 37°C (aliquot sample). Then, an additional 130 ml of 1% buffered peptone water was immediately added to the bag with the carcass (bringing the volume to 500 ml), the bag was reshaken, and the carcass and rinse were incubated for 24 h at 37°C (whole-carcass enrichment sample). Following incubation, 0.5-ml samples for the two methods were placed into 10 ml of Rappaport-Vassiliadis broth and into 10 ml of tetrathionate (Hajna) broth and incubated at 42°C for 24 h. Each broth was then streaked onto BG Sulfa agar and modified lysine iron agar and incubated for 24 h at 35°C. Suspected *Salmonella* colonies were inoculated onto triple sugar iron and lysine iron agar slants and incubated at 35°C for 24 h. Presumptive positive results were confirmed by Poly O and Poly H agglutination tests. Over the 5-week period, 13% of the aliquot samples tested positive for *Salmonella,* compared with 38% of the whole-carcass enrichment samples from the same carcasses. Recovery rates ranged from 0 of 20 samples to 4 of 20 samples for aliquot method and from 4 of 20 samples to 10 of 20 samples for the whole-carcass enrichment method over the 5-week period. These results indicate that when small numbers of *Salmonella* are expected, the sampling method has a major influence on the identification of *Salmonella*-positive carcasses.

Simmons et al. *(13)* reported that 34% of retail broilers sampled by a whole-carcass enrichment (WCE) method tested positive for *Salmonella.* This percentage is higher than the *Salmonella* incidence of 10.4% found by the U.S. Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) *(3)* for broiler carcasses sampled in processing plants. However, the results of Simmons et al. *(13)* were consistent with those of several other studies in which *Salmonella* incidence in retail poultry products was determined. Simmons et al. *(13)* suggested that the difference between their results and those obtained by the USDA-FSIS may have been due to the different sampling methods used (rinse aliquot versus WCE), to different postmortem sampling times and locations (in-plant sampling versus retail sampling), to other sampling differences (national versus regional sampling, year-round versus seasonal sampling), or to the possibility of postprocessing contamination.

In the baseline study reported by the USDA-FSIS *(1), Salmonella*-positive carcasses (20% of those sampled) were subjected to a most-probable-number (MPN) estimate of *Salmonella* numbers. The FSIS reported that <12 cells were recovered from ca. 42% of the *Salmonella*-positive carcasses and that <120 cells were recovered from ca. 45% of the *Salmonella*-positive carcasses (with reported MPNs per milliliter being multiplied by 400 to take into account the entire carcass rinse volume). Thus, for ca. 87% of the *Salmonella*-positive carcasses, 400 ml of rinse liquid contained <120 cells.

It has previously been reported that salmonellae occurring at low levels are difficult to detect *(10, 14).* For the official USDA-FSIS *Salmonella* sampling procedure, the whole carcass is rinsed with 400 ml of water, and a 30-ml aliquot of the rinse is used for *Salmonella* recovery *(2).* In view of the aliquot dilution (30 ml/400 ml, or 7.5% of the total rinse volume) and the expected small number of *Salmonella* cells per carcass, the current USDA-FSIS sampling procedure may underestimate the number of *Salmonella*-positive carcasses.

The purpose of the present experiment was to determine the effect of sampling method on the recovery of *Salmonella.* This experiment compared the recovery of *Salmonella* from a 30-ml aliquot of 400 ml of rinse with that from a WCE in 500 ml of rinse *(13)* for individual carcasses purchased in supermarkets.

MATERIALS AND METHODS

Sample collection. Twenty fresh whole broiler carcasses were purchased each week from grocery stores in northeast Georgia over a 5-week period. Only carcasses with intact packages

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TABLE 1. *Recovery of* Salmonella *from whole broiler carcasses with the use of a 30-ml aliquot of 400 ml of buffered peptone rinse (aliquot) or with the incubation of 500 ml of buffered peptone rinse and the carcass together (WCE) by week and for the entire experiment*

Week	No. of *Salmonella*-positive carcasses/no. of carcasses tested		χ^2	χ^2 *P* value
	Aliquot	WCE		
1	4/20	9/20	2.9	0.0914
2	3/20	10/20	5.6	0.0181
3	0/20	8/20	10.0	0.0016
4	4/20	7/20	1.1	0.2881
5	2/20	4/20	0.8	0.3758
Total	13/100	38/100	21.2	<0.0001

were selected. All carcasses were purchased on Mondays to ensure that birds had been slaughtered at least 3 days previously. Carcasses were selected at random from four grocery stores without regard to plant, brand, or sell-by date.

Isolation procedures. On the day of purchase, the exterior of each package was swabbed with 100% ethanol and opened with a sterile scalpel. With the use of a fresh pair of sterile gloves for each carcass, the giblets were removed and discarded. The entire carcass and the package exudate were transferred to a sterile polyethylene bag (16 by 16 in. [41 by 41 cm]) containing 400 ml of buffered peptone water (Becton Dickinson, Sparks, Md.). The bag was then vigorously shaken for 1 min, and a 30-ml aliquot was removed (aliquot sample). Then, an additional 130 ml of buffered peptone water was immediately added to the bag with the carcass, bringing the carcass rinse volume to 500 ml (400 ml − 30 ml + 130 ml = 500 ml) *(4),* and the bag was shaken again and the carcass and rinse solution were kept together in the rinse bag as previously described *(13)* (WCE sample). Both the aliquot and the WCE samples were incubated for 24 h at 37°C. For all samples, 0.5 ml of incubated rinse solution was transferred to 10 ml of Rappaport-Vassiliadis broth (Becton Dickinson) and to 10 ml tetrathionate broth (Hajna; Becton Dickinson) and incubated at 42°C for 24 h. Each broth was then streaked onto BG Sulfa agar (Becton Dickinson) and modified lysine iron agar (Oxoid, Basingstoke, Hampshire, UK) plates and incubated for 24 h at 35°C. Suspected *Salmonella* colonies were picked and inoculated onto triple sugar iron (Becton Dickinson) and lysine iron agar (Becton Dickinson) slants and incubated for 24 h at 35°C. Presumptive positive results were confirmed by Poly O (Becton Dickinson) and Poly H (Microgen, Camberley, Surrey, UK) agglutination tests.

Statistical analysis. The data are presented as numbers of *Salmonella*-positive carcasses for each method per 20 samples per week over the 5-week period. To test for differences between frequencies of *Salmonella*-positive carcasses for the two methods, a chi-square test was carried out with the use of the GENMOD procedure of SAS with a binomial distribution and a logit link function *(12).* This procedure has the advantage of comparing frequencies for paired samples (aliquot versus WCE results for each carcass).

RESULTS AND DISCUSSION

Weekly recovery rates ranged from 0 of 20 samples to 4 of 20 samples for the aliquot method and from 4 of 20 samples to 10 of 20 samples for WCE method (Table 1). Over the 5-week period, 13% of the aliquot samples were found to test positive for *Salmonella,* compared with 38% of the WCE samples from the same carcasses. This difference was significant ($P < 0.0001$). All of the carcasses testing positive by the aliquot sampling method were found to test positive by the WCE method, except for one carcass in week 4.

These results are consistent with those obtained by Surkiewicz et al. *(15),* who reported that the incubation of 270 ml of a 300-ml volume of lactose broth used to rinse poultry was four times as effective as the incubation of a 10-ml aliquot of this lactose broth in recovering *Salmonella.* Cox et al. *(5)* also cautioned against the incubation of small aliquots of rinse samples to determine *Salmonella* incidence because of the likelihood of false-negative results. The sensitivity of the WCE procedure was examined in a study by Cox and Blankenship *(4)*; these authors reported that as few as eight inoculated cells could be recovered from a carcass with the procedure.

The probabilities that at least one salmonella CFU will be present in 30-ml aliquots from 400-ml rinse samples containing various numbers of salmonella CFU are shown in Figure 1. This probability is calculated as $1 - (1 - 30/400)^n$, where n is the number of salmonellae suspended in the 400-ml volume. The probability that any salmonellae in the aliquot will survive cultural procedures and be detected

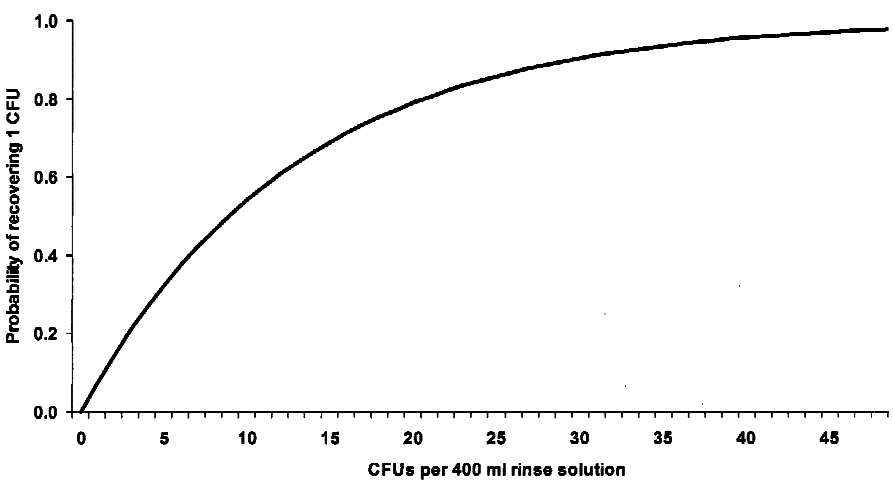

FIGURE 1. *The relationship between the number of CFU present in a 400-ml rinse sample and the probability of recovering 1 CFU from a 30-ml aliquot of a 400-ml rinse sample on the basis of the formula* P = 1 − (1 − 30/400)n, *where* n *is the number of CFU present in a 400-ml rinse sample.*

in the assay is another matter, but the graph shows that the probability that salmonellae will be present in a small aliquot of a total sample declines relatively sharply as the number of salmonellae in the total volume decreases. Differences between the aliquot and WCE procedures used in this study are increased by the fact that rinse sampling does not recover 100% of bacteria, including salmonellae, that are on a carcass *(7–9, 11).* If rinse sampling recovers salmonellae from a carcass, it is likely that still more salmonellae are on the carcass.

The *Salmonella* incidence obtained with the WCE method (38%) is consistent with the levels obtained in many earlier surveys and with the incidence of 34% obtained by Simmons et al. *(13),* so there is no reason to think that the incidence or the distribution of *Salmonella* in the present experiment was unusual. Whether these results can be compared directly with those obtained by the FSIS through rinse sampling immediately after chilling is unknown. The FSIS sampled carcasses in the plant immediately after ≥45 min of immersion chilling. Thus, the carcasses would have been subjected to exhaustive ''rinsing'' during this immersion chilling immediately prior to rinse sampling, which may have resulted in a lower-than-expected level of recovery. The number of salmonellae recovered from sequential 1-min rinses of chicken carcasses declines steadily *(7–9),* as do numbers of several types of bacteria recovered through the repeated sampling of inanimate surfaces *(6).* In this experiment, as well as in the preceding work *(13),* rinse sampling took place at least several days after chilling, and the partial recovery or shedding of salmonella cells might have occurred during this interval. However, the recovery incidences do not support this hypothesis.

The results of the present study indicate that the sampling method has an effect on *Salmonella* recovery when expected numbers of *Salmonella* per carcass are small. These results also indicate that the difference between the results of Simmons et al. *(13)* and those of the FSIS *(3)* may be due in part to different sampling methods. The carcass sampling method currently used by the FSIS may be underestimating the incidence of *Salmonella* on poultry, especially if current industry practices used to meet current food safety regulations *(2)* are reducing the actual number of *Salmonella* per carcass. It can be seen from Figure 1 that the probability of obtaining a positive result with the 30-ml aliquot method if the number of CFU present in the rinse is >20 would be >80%. The results of this experiment suggest that the number of salmonella CFU present in the 400-ml rinse sample was too small for detection by the 30-ml/400-ml aliquot method, given the method's practical sensitivity.

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